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DASA 1286

TECHNICAL PROGRESS REPORT

DEFENSE ATOMIC SUPPORT AGENCY

WASHINGTON 25, D.C.

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Title: The Effect of Total Body Irradiation on Immunologic Tolerance of Bone Marrow and Homografts of Other Living Tissue

Report Period: Feb. 1, 1961 - Jan. 31, 1962

Contract Number: DA-49-146-XZ-032
Modification No. 1

Supported by: Defense Atomic Support Agency
Washington 25, D.C.

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This investigation has its origin more than 10 years ago in a clinical proposal that total body irradiation might have a role similar to that of chemotherapy in the treatment of patients with disseminated cancer. Such treatment should have certain advantages: 1) irradiation has demonstrated value in inducing regression in a wide variety of types of cancer, 2) precise dosimetry at target sites, not possible with drugs, would allow greater accuracy in correlating radiation delivered and effect produced, 3) such control of dosage would diminish side effects and complications of treatment by allowing consistent observance of tolerance limits, 4) treatment could be offered with a minimum of discomfort to patients. These advantages have been demonstrated but total body irradiation has not been widely used because the therapeutic armamentarium for advanced cancer has been saturated with an ever-increasing number of chemotherapeutic agents made available through the therapeutic trials program. Nevertheless, experience gained has offered valuable basic information on the effects of irradiation on the disease itself and on systemic tolerance.

The desirable dose in cancer therapy, for radiation or drugs, is the maximum tolerable dose. Therefore, it is of primary importance to evaluate the factors involved in tolerance. In this study the intent has been to restrict the dose of total body irradiation to that which can be tolerated without resort to planned reliance on supportive measures. This permits observation of the natural course of radiation effect and recovery while reserving supportive measures as a safeguard against excessive response in ill patients. Such supportive measures have been necessary in some patients, isolation, antibiotics, steroids, transfusions and autologous marrow

transplants. As the dependability of these benefits becomes more firmly established, the level of therapeutic dose used may be raised.

This report deals with 5 aspects of the problem of total body irradiation.

I. Clinical records of patients receiving therapeutic total body irradiation.

II. Experimental observations on the effect of iliac shields in total body irradiation of dogs.

III. Experience in maintaining an autologous bone marrow bank.

IV. A study of granulocyte proliferation as a basis for predicting the degree of bone marrow depression following total body irradiation.

V. Future plans; Clinical studies, laboratory studies, literature review, bone marrow bank and bone marrow studies.

Part I. Observations on patients receiving therapeutic total body irradiation.

In this study a total of 106 patients have received total body irradiation of whom 8 have been treated during the past year. Since most of these patients have a diagnosis of cancer at the disseminated stage, the period of observation is apt to be brief. However, some patients have shown a very satisfactory clinical response to treatment, providing prolonged periods of observation. While it is intended that the dose range of total body irradiation utilized in this series would not be incapacitating, it is always difficult to distinguish the non-specific effects of radiation from the symptoms which mark the course of the advanced cancer patient and the bone marrow depression which may be associated with the anemia of cancer or the effects of prolonged chemotherapy. A summary of 106 patients receiving therapeutic total body irradiation is given in Table 1.

Table 1. Total Body Irradiation
Baylor Series - 106 Patients

Number known dead	81
Number living	17*
Number lost	8

*Includes 4 current patients

Details of exposure for the eight patients receiving total body irradiation during the past year are outlined in the following case histories along with observations to date.

T.B.I. No. 99 F.F. Age 40 C/M

Date	Day	
1/15/61		Generalized massive lymphadenopathy, enlarged spleen and liver with retroperitoneal mass, and right orbital involvement with ptosis of eye lid. Biopsy of inguinal node - Path. report: lymphosarcoma, followed by infection at site of biopsy with chronically draining sinus.
2/23/61	0	Total body irradiation 150r/6 days (50r x 3) 2 m v. Radiotherapy (200r) to left axillary mass No nausea or vomiting
3/1/61	6	Partial urinary obstruction due to multiple bladder and ureteral calculi progressing to complete urinary shutdown.
3/12/61	17	Normal urinary output following supportive G.U. treatment: Moderate regression of lymphadenopathy.
3/18/61	23	Died of disease.

Comment:

It would be difficult to find a patient with more advanced and widespread disease than this one. However, bone marrow depression following total body irradiation was only moderate (W.B.C. 4,550 on day 23). Despite improvement in G. U. symptoms and regression of nodes, condition deteriorated and death occurred on the 23rd day of observation. Autopsy showed involvement of all lymph nodes including those in para-aortic region, and kidneys. There was no evidence of radiation change except that shown clinically by reduction in size of lymphadenopathy.

T.B.I. No. 100 W.P. Age 48 W/M

Date	Day	
5/18/59		Weakness, easy fatguibility, anemia - 2 years
6/1/59		Exploratory laparotomy - retroperitoneal mass 15 cm. in diameter; extension and satellite masses involving all abdominal structures Biopsy - Path. report: lymphosarcoma, reticulum cell type.
6/16/59		Radiotherapy 1800r/16 days, 20 x 24 cm. abdominal field. Moderate hematopoietic depression (W.B.C. 3,200/10 days) and disappearance of abdominal mass.
3/20/61		Dyspnea, superior mediastinal mass Radiotherapy 1500r/4 days with complete disappearance of mass.
June 1961		Generalized lymphadenopathy - HN_2 Moderate regression
9/19/61		Radiotherapy 750r/3 days to right testicular mass and recurrent mediastinal nodes
9/21/61		Bone marrow aspiration, 200 cc. banked
9/22/61	0	Total body irradiation 100r/1 day, 2 m v. No nausea or vomiting Moderate regression of all lymphadenopathy
10/13/61	21	W.B.C. 4,250. Bone marrow re-infused without incident.
10/25/61	33	Lowest point of bone marrow depression (Hgb. 9.3; W.B.C. 1,450; platelets 108,000)
11/14/61	53	Widespread disease with jaundice, recurrent kidney mass and urinary obstruction.
11/22/61	61	W.B.C. 6,050; platelets 474,000.
12/7/61	76	HN_2
12/18/61	87	Died of disease

Comment:

Although at first the tumor responded to radiotherapy, intervals between treatment and recurrent tumor gradually decreased. The disease progressed relentlessly to involve all organs and systems from scalp to lower extremities. Total body irradiation 100r/1 day failed to influence the course of the disease but because of previous large field irradiation and the poor condition of the patient, a protracted course was not feasible. Hematopoietic depression following total body exposure was moderate with adequate recovery by day 61. Depression of white blood cell count was greater than ordinarily occurs with a single exposure of 100r, presumably due to bone marrow involvement by lymphosarcoma even though a satisfactory recovery of both white blood cells and platelets occurred.

T.B.I. No. 101 H.A. Age 74 C/M

Date	Day	
5/25/59		Generalized lymphadenopathy, multiple skin nodules and painful mass on lateral aspect of right knee for 3 months. Hypertensive cardiovascular disease with angina, 7 years. Biopsy of skin lesion and node - Path. report: lymphosarcoma, lymphocytic cell type. Cytoxan with disappearance of many skin nodules.
8/8/61		Necrotic mass on right knee Anemia (Hgb. 9.8 gm.) with marked leukocytosis (WBC 27,000) Radiotherapy 1000r/23 days to knee with appreciable shrinkage of mass.
8/24/61		Weakness and fever. HN_2 with poor response
9/28/61	0	Lymphadenopathy progression, patient semi-comatose W.B.C. 36,000 Total body irradiation - 50r/1 day No immediate reaction
10/10/61	12	W.B.C. 30,300 Died of disease

Comment:

Because of prolonged prior treatment with chemotherapy, caution in the use of total body irradiation was indicated. Experience has shown that 50r total body irradiation may offer relief in particularly sensitive lesions but produces practically no effect on peripheral blood count. In this patient all the factors that might intensify radiation reaction were present (advanced age, poor condition, anemia, previous treatment by chemotherapy and partial body irradiation, yet the leukocytosis was not influenced by total body irradiation.

T.B.I. No. 102 L.E. Age 61 W/M

Date	Day	
9/1/61		Craniotomy for removal of meningioma 5th postoperative day - massive G. I. bleeding
9/9/61		Exploratory laparotomy for mass involving antrum and duodenum, adhering to gallbladder - non-resectable Biopsy - Path. Report: lymphosarcoma, lymphocytic cell type.
9/11/61		Radiotherapy 300r/1 day to abdomen
9/22/61		Blood studies, Rebuck test and bone marrow aspiration (305 cc. marrow aspirated, processed and stored) in preparation for total body radiation. W.B.C. 10,800
10/2/61	0	Total body irradiation 150r/1 day 2 m v No nausea or vomiting
10/10/61	8	Radiotherapy, large abdominal field (15 x 15 cm.) 1000r/5 days
10/19/61	17	Much improved, no G.I. symptoms; W.B.C. 5,400
12/1/61	60	Bone marrow recovery complete - W.B.C. 8,950 Patient feels well, appetite good, no palpable nodes or masses.

Comment:

The patient did remarkably well, tolerating radiation without reaction and with excellent therapeutic benefit. Only moderate hematopoietic depression was noted, recovery was spontaneous and re-infusion of bone marrow was not indicated. Prior to total body irradiation, there was no clinical evidence of anemia and the patient had received no prior treatment by chemotherapy; This may be the most important factor in rate and degree of recovery following single exposures at this level.

T.B.I. No. 103 D.H. Age 32 C/M

Date	Day	
6/13/61		Generalized lymphadenopathy - HN_2 followed by slight remission of symptoms
9/21/61		Generalized lymphadenopathy, fever, severe low back pain. Biopsy of lymph node - Path. report: Hodgkin's disease
10/4/61		Bone marrow aspiration attempted - unsuccessful Anemia, W.B.C. 4,000; platelets 98,000
10/6/61	0	Total body irradiation 150r/18 days (50r x 3) 2 m v followed by marked regression of nodes
10/12/61	6	Continued fever, ? infection; antibiotics started.
11/7/61	32	Fever associated with Hodgkin's disease, antibiotics discontinued and steroid therapy started.
11/10/61	35	Severe marrow depression; W.B.C. 644, platelets 20,000
11/22/61	47	Beginning marrow recovery; W.B.C. 1,697, platelets 24,000
11/27/61	52	Evidence of continued bone marrow recovery, W.B.C. 2100 platelets 36,000, but condition continued to deteriorate.
11/28/61	53	Expired

Comment:

This patient's condition was poor on admission. There was widespread lymphadenopathy, anemia secondary to disease and to previous treatment with HN_2, and severe low back pain and fever indicating probable involvement of bone and marrow. It is believed that these factors were responsible for failure to obtain marrow when aspiration was attempted. Antibiotics and steroid therapy may have contributed to the prolonged depression and the correspondingly slow recovery rate. It is probable, too, that 150r/18 days is less well tolerated than 150r/1 day (as in the former patient, T.B.I. No. 102).

T.B.I. No. 104 J.D.C. Age 37 W/M

Date	Day	
9/9/61		Epigastric pain with hematemesis - 5 years; jaundice associated with enlarged liver, separate abdominal mass with cervical, axillary and inguinal lymphadenopathy - 3 months Biopsy - Path. report: lymphosarcoma, follicular type
10/14/61		Radiotherapy to abdomen 100r/1 day
10/17/61		Patient very ill; blood studies and bone marrow aspiration (320 cc. stored) in preparation for total body irradiation
10/20/61	0	Total body irradiation 200r/7 days (100r x 2) 2 m v Nausea and vomiting followed second exposure; this reaction attributed to drugs
10/31/61	11	Regression of nodes, abdominal mass much smaller, jaundice cleared
11/12/61	23	Headaches and abdominal discomfort due to non-functioning kidney, cleared with continued shrinkage of abdominal mass
11/17/61	28	Hematopoietic depression - Hgb. 6.6, W.B.C. 866, platelets 14,000 Re-infusion of bone marrow, isolation to avoid infection
11/28/61	39	Continued depression, 2 transfusions 1000 cc. each/8 days
12/4/61	45	Beginning recovery, W.B.C. 2,150, platelets 80,000
12/27/61	68	Remarkable improvement; looks well and feels well. Abdominal mass still palpable but considerably reduced, no other lymphadenopathy. Hgb. 11.2, W.B.C. 3,900, platelets 212,000.

Comment:

Therapeutically, the effect of 200r/7 days (100r x 2) has been excellent. Because of prolonged illness with general debilitation and anemia (Hgb. 8.8 gms. on admission), the severe bone marrow depression was anticipated. The patient became ambulatory and at 68 days, there was no clinical evidence of radiation change while the blood picture was better than at the time of admission. This remarkable recovery may be attributed to the therapeutic effect of total body irradiation, transfusions, bone marrow re-infusion, or a combination of these factors.

T.B.I. No. 105 J.R. Age 43 C/M

Date	Day	
5/8/59		Admitted for treatment of unexplained anemia Diagnosis could not be established
5/18/60		Cough, chest pain, generalized lymphadenopathy and splenomegaly. Biopsy - Path. report: lymphocytic cell lymphosarcoma HN_2 without benefit
7/12/60		Radiotherapy to mediastinal mass 2400r/11 days with relief of cough and chest pain
10/23/61		Progressive lymphadenopathy with recurrent mediastinal mass and bronchial obstruction Hgb. 10.9, W.B.C. 3,700, platelets 192,000 Bone marrow aspiration attempted, insufficient marrow obtained
10/30/61	0	Total body irradiation 200r/19 days (50r x 4) 2 m v No nausea or vomiting
11/28/61	29	Considerable reduction in size of nodes W.B.C. 1,560, platelets 66,000
12/27/61	59	No complaints; looks and feels well. W.B.C. 2,600, Hgb. 8.5, platelets 212,000

Comment:

Because of previous irradiation and chemotherapy, rather severe depression was anticipated following total body irradiation 200r/19 days. No supportive treatment of any kind was offered throughout the period of depression and recovery. Except for decrease in size of nodes, there is no evidence of radiation reaction or change.

T.B.I. No. 106 W.Z. Age 32 C/M

Date	Day	
June 1960		Diagnosis of Hodgkin's disease established. Radiotherapy 2500r to cervical nodes, and in August 1960 850r to posterior pharyngeal region. HN_2 followed by painful laryngeal edema Edema gradually subsided, only slight regression of nodes
8/2/61		Cervical and supraclavicular adenopathy Radiotherapy 900r/12 days with moderate regression
11/24/61		Large bilateral cervical masses, fever 104° liver enlarged Nasopharyngeal involvement with bleeding and dysphagia
12/8/61		Bone marrow aspiration and blood studies in preparation for total body irradiation. 325 cc. marrow aspirated and stored Hgb. 10.8, W.B.C. 5,850, platelets 314,000
12/15/61	0	Total body irradiation 300r/14 days, 2 m v Vomiting followed first exposure, attributed to bleeding No further nausea or vomiting
1/8/61	24	No change in size of cervical masses, no further bleeding Hgb. 11.4, W.B.C. 1,220, platelets 36,000 Condition deteriorating

Comment:

During the course of total body irradiation 300r/14 days (100r x 3) the patient developed an abscessed tooth requiring extraction; there were no complications following this procedure. On day 24 there was little or no regression of lymphadenopathy. Patient is following a downhill course but bone marrow depression is not alarming.

Summary

Diagnosis, details of exposure, course and survival for these 8 patients are summarized in Table 2.

The pattern of peripheral white blood cell depression has been documented repeatedly in earlier patients. In general terms it may be said that, irrespective of dose, there is consistency in the time of maximum depression. The rate of fall and the degree of depression, the rate of recovery and the degree of recovery bear some relation to dose but the severity of depression and time of recovery have not shown such consistency as to permit a confident prediction of the effect that may result from a given dose in any one patient.

This may be reasonably explained by the effect of chronic disease, bone marrow invasion by cancer, and the latent effect of previous chemotherapy. Two patients in the group died on days 12 and 23 post-irradiation, before the effect of exposure could be evaluated. Response of white blood cell count and platelets for the remaining 6 patients is shown in figures 1 - 4.

Table 2. Total Body Irradiation - 8 Patients

No.	Pt.	Diagnosis	Prior Treatment	Exposure	N. & V.*	Depress.	Recovery	Survival
99	F.F.	Lympho-sarcoma	0	150r/6days (50rx3)	0	++/23d.		Died of dis. Day 23
100	W.P.	Lympho-sarcoma	Radiation (3 courses) HN_2	100r/1 day	0	+/35d.	Day 61	Died of dis. Day 87
101	H.A.	Lympho-sarcoma	Cytoxan Radiation	50r/1 day	0	0		Died of dis. Day 12
102	L.E.	Lympho-sarcoma	Radiation	150r/1day	0	++/33d.	Day 60	Living Day 74
103	D.H.	Hodgkin's disease	HN_2	150r/18day	0	+++/35d.	Day 51	Died of dis. Day 53
104	J.D.C.	Lympho-sarcoma	Radiation	200r/7days (100r x 2)	+	++++/39d.	Day 68	Living Day 78
105	J.R.	Lympho-sarcoma	HN_2 Radiation	200r/19days (50r x 4)	0	+++/29d.	Day 59	Living Day 70
106	W.Z.	Hodgkin's disease	Radiation (3 courses) HN_2	300r/21day (100r x 3)	+	++++/25d.	Current	Current

*Degree of nausea and vomiting, and bone marrow depression

None 0
Slight +
Mild ++
Moderate +++
Severe ++++

Fig. 1

PERCENT CHANGES IN W.B.C. FOLLOWING VARYING EXPOSURE TO TBI (HUMANS)

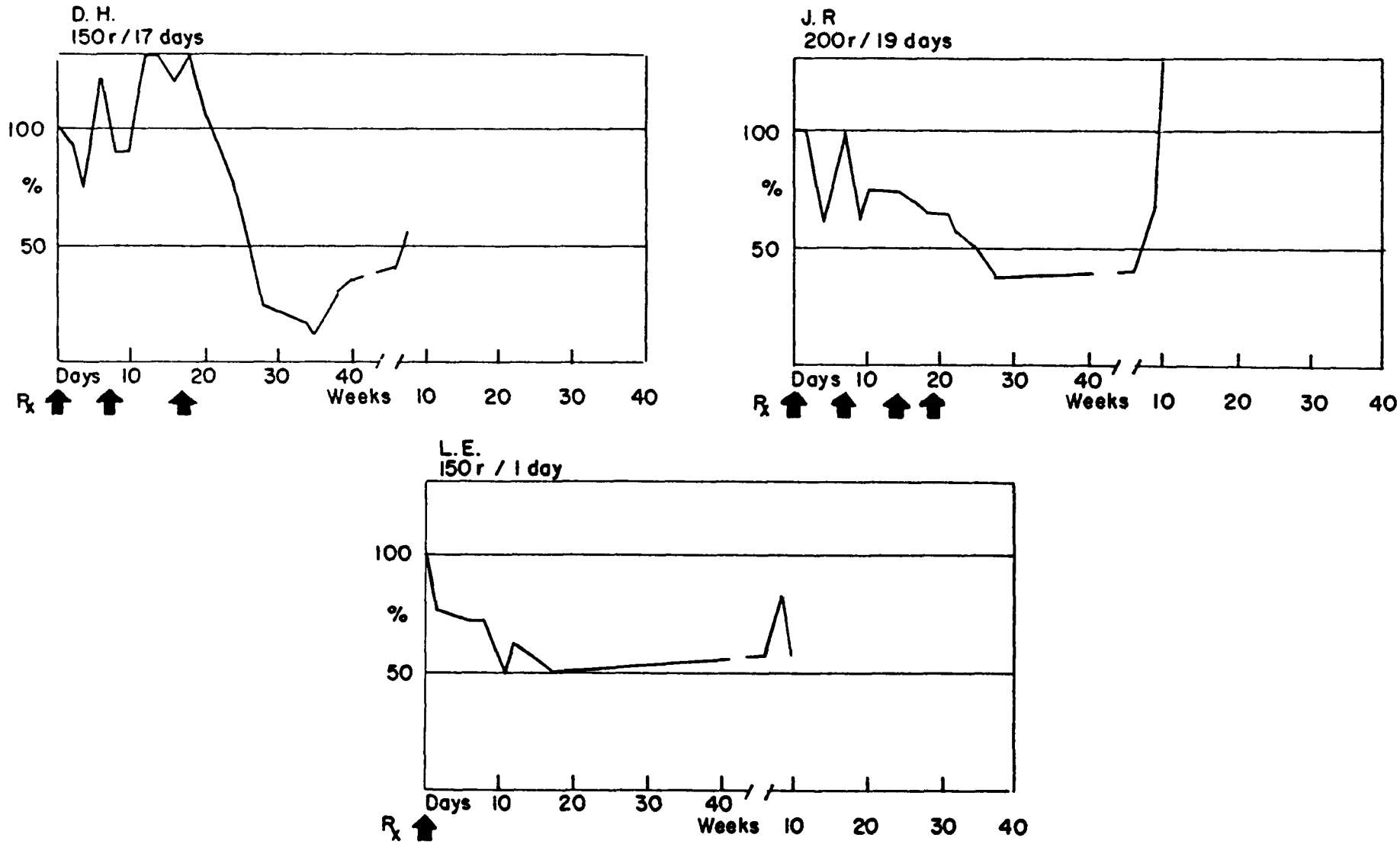


Fig. 2

PERCENT CHANGES IN WBC FOLLOWING VARYING EXPOSURE TO TBI (HUMANS)

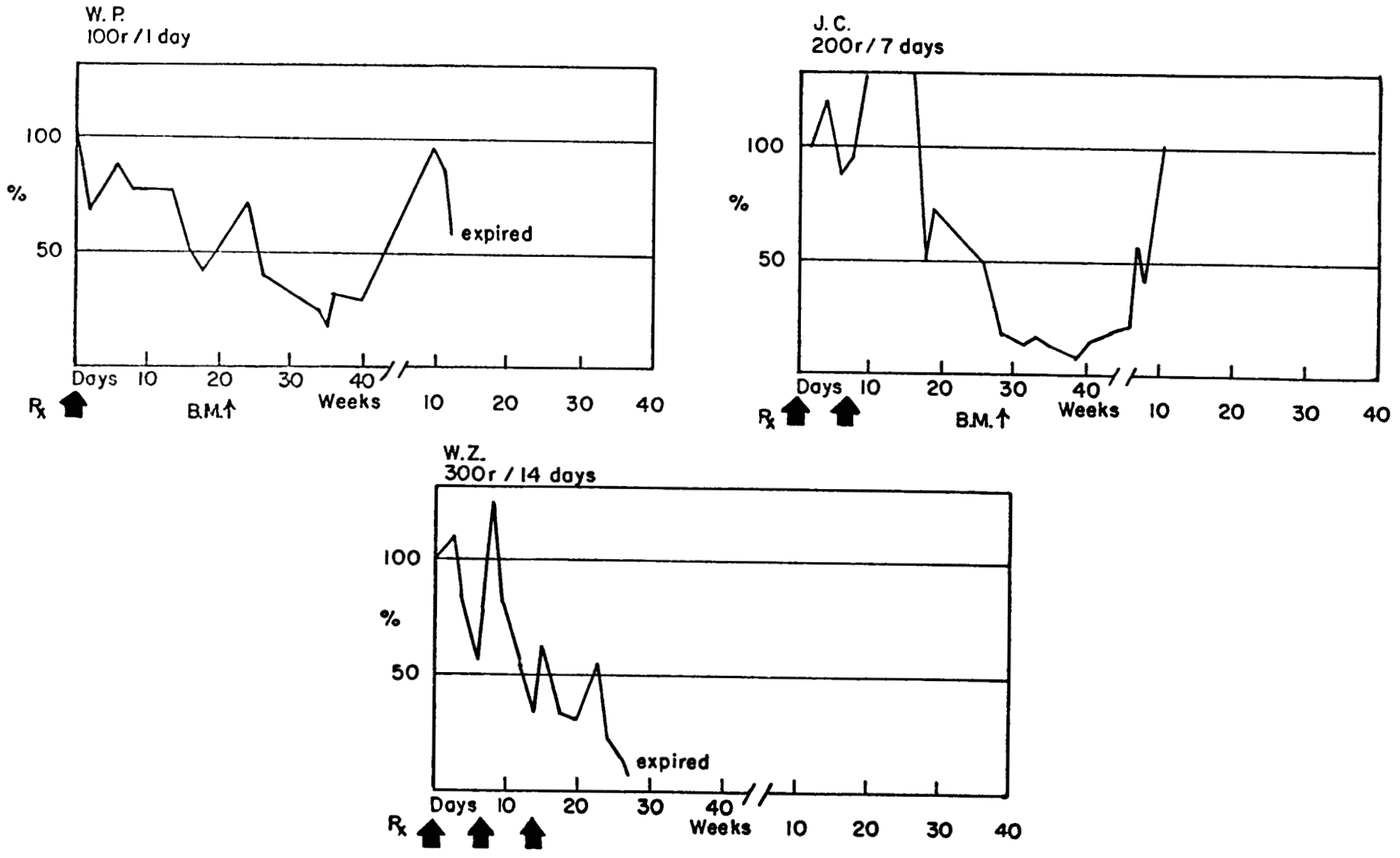


Fig. 3

PERCENT CHANGE IN PLATELETS FOLLOWING VARYING EXPOSURE TO TBI (HUMANS)

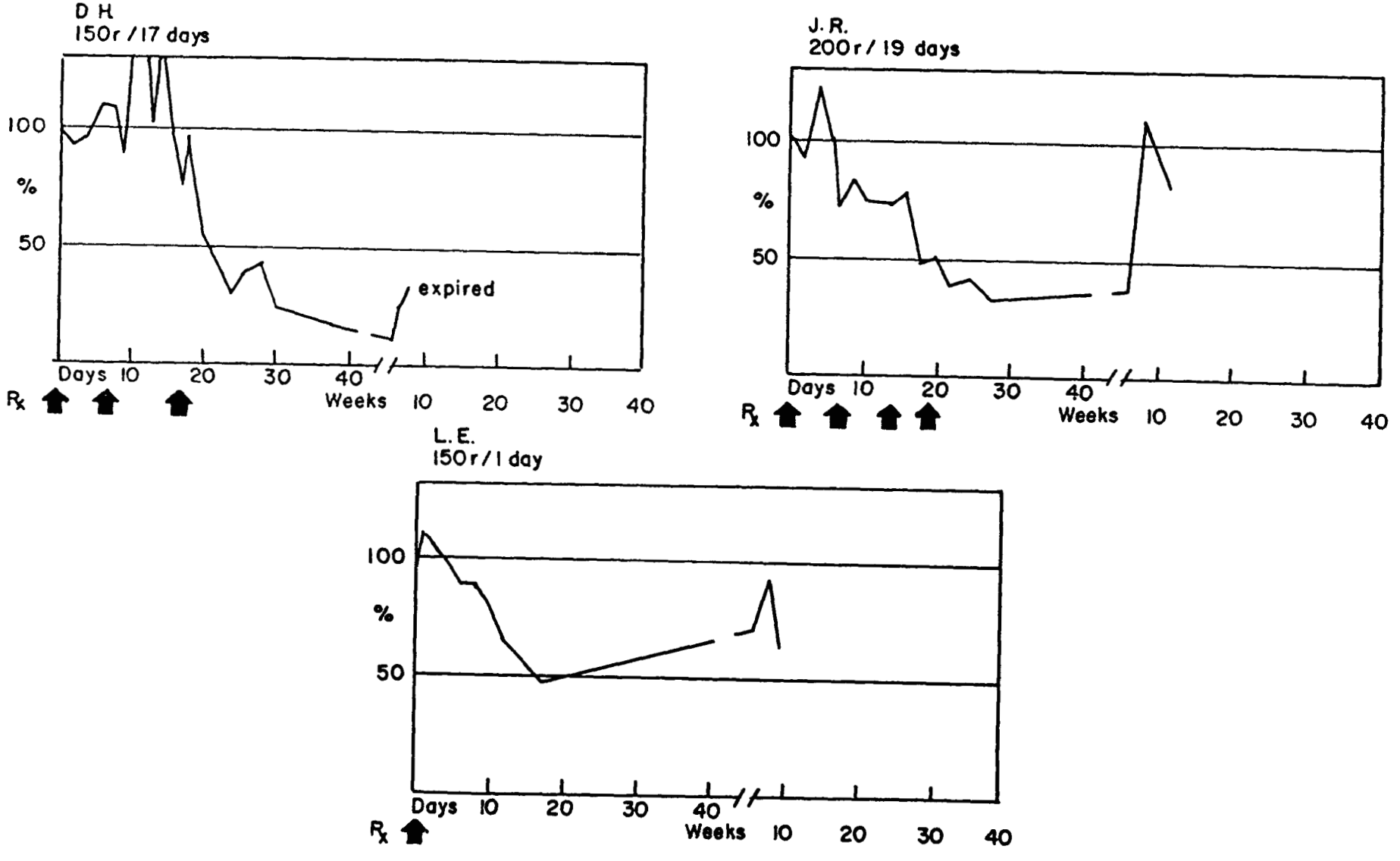
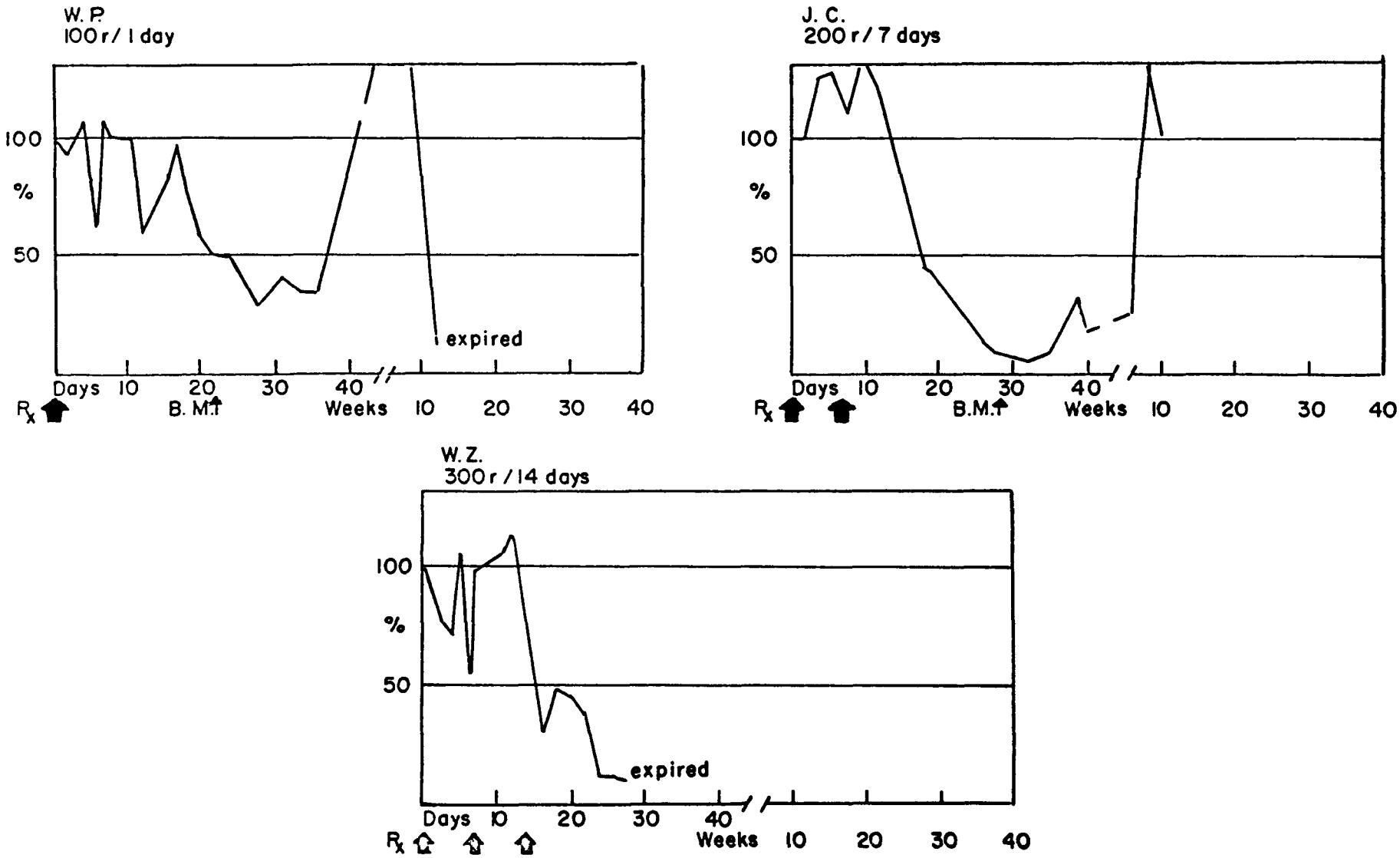


Fig. 4

PERCENT CHANGE IN PLATELETS FOLLOWING VARYING EXPOSURE TO TBI (HUMANS)



Part II. Animal Experiments

Previous reports have indicated that for the circumstances of irradiation in this department, the LD-100 for dogs was 700r in a single exposure. In this technique the target axis distance was 223 cm., the animal was centered to the axis; half the exposure was given to the right lateral aspect and half to the left lateral aspect of the animal. The dose rate at axis was 13r per minute. In the dose range of 600 to 800r all animals died at approximately two weeks (minimum 8 days, maximum 17 days). In all animals dying in this period, death was due to bone marrow depression. It is noted that when animals were irradiated from one side only, two of four dogs exposed to 700r survived, but all animals receiving 800 to 1000r died in approximately two weeks.

It was previously reported that when an iliac shield was added to the same conditions of irradiation, survival was obtained with an exposure of 1200r. Four dogs so treated have survived from 1 to 1½ years in good health. Following 1600r with full iliac shield,all animals died with severe gastroenteritis.

An experiment was undertaken to study the mechanism of bone marrow injury and recovery. In two animals the method of irradiation was to expose the animal to 1000r with protective iliac shields in place, then 14 days later 1000r was delivered to the iliac region that previously had been protected. This procedure was reversed with two additional animals who first received 1000r to the iliac region and 14 days later were exposed to 1000r total body irradiation with the iliac region shielded. All four

animals have survived from 14 to 18 months. These experiments are the basis for the observations on granulocyte proliferation described in Part IV.

Part III. Bone Marrow Bank

This study was undertaken with a view to determine: 1) the degree of protection offered by bone marrow transfusion following high doses of total body irradiation, 2) the effect of total body irradiation on inhibition of immune response, and 3) whether high doses of total body irradiation and supportive measures permit tolerance of organ and tissue transplants.

The first step involved the aspiration and storage of marrow prior to total body exposure and re-infusion when indicated by severity of depression following exposure. Under local anesthesia and sedation, or general anesthesia, marrow is aspirated from the iliac crest, using multiple sites if needed. Approximately 50 cc. of marrow is drawn into a siliconized syringe containing 5 cc. of a solution containing 20 mg. of heparin to 100 cc. of Earl's solution. The diluted marrow is distributed as 5 cc. aliquots in siliconized, screw-capped test tubes. Because the cell count per cc. of marrow varies considerably, a small sample from each syringe filled with marrow is saved for leukocyte count and viability determination; an attempt is made to collect 5 to 6 x 10^9 nucleated marrow elements. To each test tube containing marrow, 5 cc. of 30% glycerol in modified Earl's tissue culture solution is added. A sample is taken for culture to be certain the technique has been sterile. The remainder is placed in the freezer immediately and frozen at the rate of 1° per minute to minus 20°, and then at approximately 2° to 3° per minute to minus 79° C.

Marrow is thawed by allowing tubes to stand at a temperature of zero

to 4° C. As soon as the marrow liquifies, 5 cc. of Earl's 30% dextrose is added by means of a 13 guage siliconized needle with a 50 cc. syringe and mixed by inversion. A sample of the marrow plus the glucose mixture is taken for cell count, differential count and viability determination. Viability is determined by adding 0.3 cc. of cold 2% tryptan blue in Ringer's solution to 0.3 cc. of the marrow plus dextrose. The stained cells are considered dead, the unstained cells are viable. Hemolysis is determined by taking a hematocrit and noting whether the serum has changed color. Culture for sterility is carried out at monthly intervals during storage and prior to re-infusion.

Bone marrow aspiration, storage and re-infusion was carried out first in experimental animals. Of 68 animals in this experiment, 30 received re-infusion immediately following total body irradiation without storage while in 38 dogs, marrow as stored and re-infused at a later date. Extension of this work to humans required some alteration in technique, the major problem being inability to obtain sufficient marrow for processing, storage and re-infusion. This was due, in part, to technical difficulty, and in some instances to individual characteristics of the patients. For example, the procedure may be unsuccessful in patients having severe bone marrow involvement by disease or in those with severe bone marrow depression following prolonged chemotherapy. With more careful selection of patients, these difficulties are being overcome and an adequate amount of marrow is now obtained almost routinely. In the meantime, certain aspects of technique have been simplified so that less time is required for a single procedure of aspiration and processing.

Table 3 summarizes a group of patients who underwent bone marrow aspiration after the technique had become fairly standardized. Six of the 14 patients have received total body irradiation. The clinical course for each of these six patients is outlined in Part 1.

Because of expanding activities, more storage space was needed; a new sub-freezing unit was ordered and is now being installed. This unit, along with improvement in technique, will permit an increase in the number of patients in the study as well as more complete observations on each patient.

At present, the major function is to hold in reserve stem cells for repopulation of marro depleted by total body irradiation. In patients receiving single exposures or those receiving therapeutic agents which are immediately fixed in tissue, the marrow can be returned within 24 hours. Thus, beginning depression and beginning function of transfused marrow, theoretically, can occur simultaneously. However, when protracted exposures are used, the problem is more complex; it suggests that re-infusion might be most beneficial if returned in divided amounts, perhaps proportional to the time of exposures, or proportional to the cumulative effect of protracted exposures. Some of the possibilities for investigating an appropriate time for bone marrow transfusion by means of the granulocyte proliferation study are discussed in Part IV of this report.

Table 3. Bone Marrow Bank - Summary of 14 Patients

Patient	Diagnosis	Cell count (billions)	cc.	Disposition
H.G.	Renal cell Ca.	45.1	360	All marrow returned; 90% viability
J.D.	Mesothelioma	65.6	320	Stored; to be re-infused when indicated
J.H.	Ca. of duodenum	82.8	430	" " " " " "
J.H.	Ca. of pancreas	43.7	310	Stored, patient died; saved for viability tests
F.G.	Ca. of lung	27.7	250	" " " " " " "
A.L.	Ca. of lung	36.0	300	" " " " " " "
W.P TBI. 100	Lymphoma	38.0	250	One-half of marrow returned; 90% viability
L.E. TBI. 102	Lymphoma	56.0	305	Stored; to be re-infused when indicated
D.H. TBI 103	Hodgkin's dis.	Dry tap		HN_2 8 weeks prior to attempt with resultant depression and progressive disease
J.B.	Sq. cell Ca.	35.8	340	Stored; to be re-infused when indicated
J.D.C. TBI 104	Lymphoma	73.0	340	One-half of marrow returned; 90% viability
J.R. TBI. 105	Lymphoma		9	Technical difficulty
W.Z. TBI. 106	Lymphoma	44.4	325	Stored, patient died; saved for viability tests.
D.B.	Ca. of testis	49.0	140	Stored, patient died; saved for viability tests.

Part IV. Granulocyte Proliferation Study

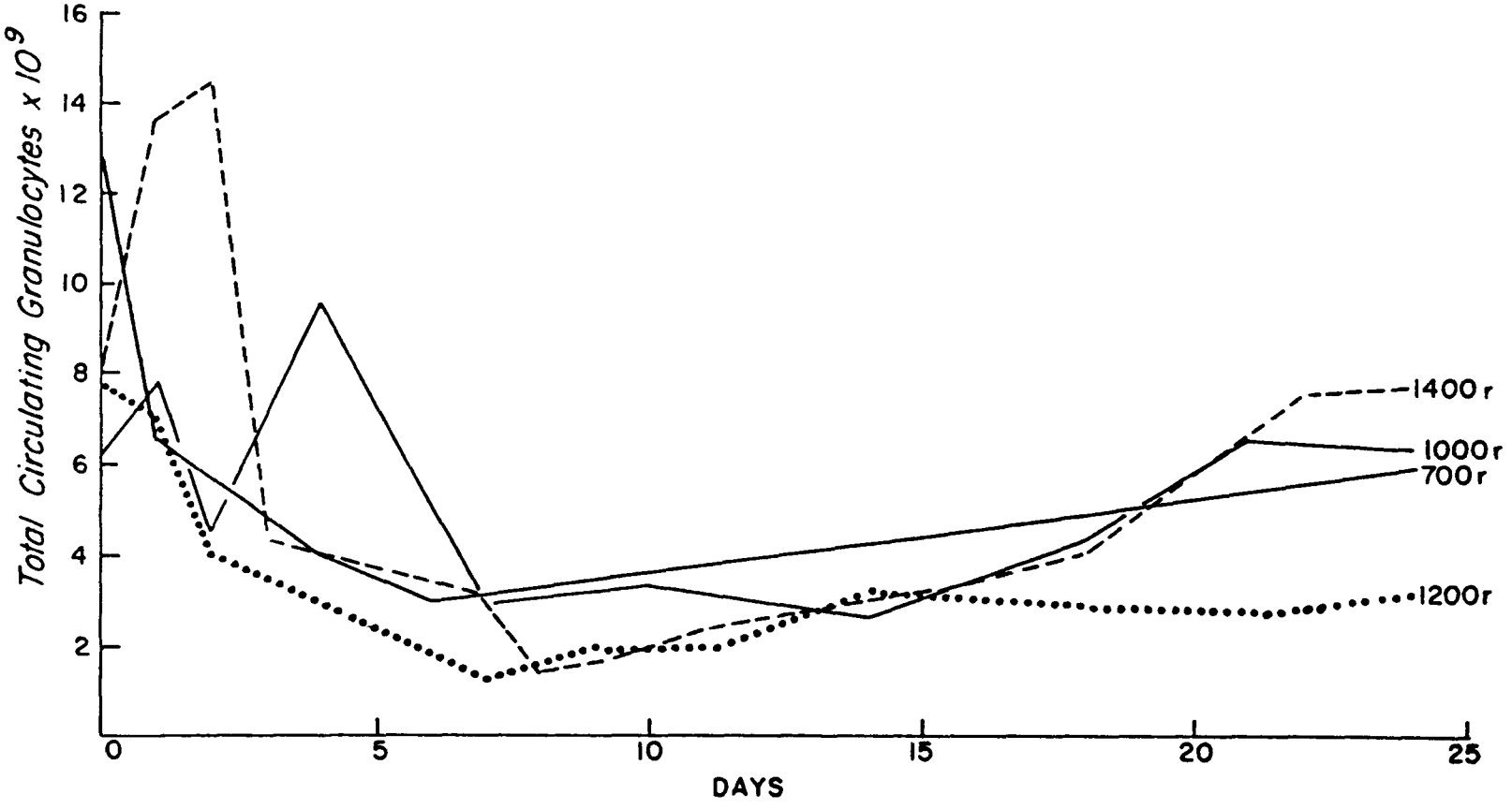
Under conditions of exposure in this laboratory, the LD-100 for dogs has been established at between 600 and 700r, but by shielding iliac marrow, survival can be obtained with exposures of 1200r - 1400r. All deaths following total body exposures above this level were due to gastro-intestinal tract syndrome; below this level, deaths were caused by bone marrow depression (see Part II). An Analysis of events in bone marrow depression and recovery was undertaken in order to clarify the relation between exposure to radiation and the peripheral blood count response. If the mechanisms involved could be defined, it might be possible 1) to predict effects when amounts of exposure were known or, 2) to determine amount of exposure when the course of marrow depression was analyzed. It seems probable that the possibilities of the peripheral blood count as a quantitative index of radiation exposure and effect have not been exhausted in the light of experimental data on compartments of granulocyte precursors.

Bone marrow depression is reflected in the peripheral blood cells originating in bone marrow, red blood cells, platelets and granulocytes. The time at which changes in these lements will become evident is a function of the rate of production and the survival time. Granulocytes, having the shortest life span (up to 4 days), would be the first to reflect a change due to irradiation.

Following exposures ranging from 700r to 1400r with iliac marrow protected by shields (see Part II), the circulating granulocytes are plotted to illustrate changes in degree and time of depression, and rate of recovery (Fig. 5). The depression following 1200r and 1400r is similar and somewhat lower than that following 700r and 1000r. The time of lowest depression

Fig. 5

CHANGES IN TOTAL CIRCULATING GRANULOCYTES FOLLOWING TOTAL BODY IRRADIATION WITH ILIAC SHIELDS (DOGS)



was the same in all 4 animals and the rate of recovery suggests a constant, almost linear increase in cells. Thus, the degree of depression seems related to amount of exposure but the time of depression and rate of recovery are not. The linear recovery suggests a consistent increase in the early primitive cells independent of dose within the range of exposures used. The increase may be by self-duplication or by some feed-in system. The simplest mechanism would be a self-perpetuating linear increase in early stem cells. Recovery may be due to: 1) hyperproliferation of the protected marrow, or 2) proliferation of surviving stem cells in the irradiated marrow. Destruction of the protected marrow by local irradiation at some time during the recovery period should indicate which process is responsible for recovery. If the first process is responsible, there would be a complete loss of recovery; if the second were true, recovery would be continuous. If both processes are responsible for recovery, a quantitative estimate of the contribution of each could be obtained.

Accordingly, observations were made on two groups of dogs. In the first, two animals received 1000r total body irradiation with iliac shields in place; 14 days later, the shielded area of the pelvis received 1000r. In the second group, two animals received 1000r local irradiation to the pelvis; 14 days leter these animals received 1000r total body irradiation with the pelvic marrow shielded.

In the first group there was the expected depression (days 6 - 10) with recovery to day 14. At this time the previously protected area of the iliac crest was exposed to 1000r iocal irradiation and this was followed by immediate depression of circulating granulocytes to zero concentration

by day 18. This response indicated that recovery to day 14 must be due to hyperproliferation of the protected marrow spared during total body exposure. Recovery from zero concentration began about day 19-20, only 5 - 6 days following local irradiation to the iliac crest and too soon to be attributed to proliferation of iliac crest marrow. This recovery is believed to be the result of proliferation of stem cells surviving in marrow exposed to total body irradiation.

In the second group there was 50% depression of total granulocytes following local irradiation to the iliac crest marrow. Recovery began about day 6 - 10 and continued to day 14 when 1000r total body irradiation was given with iliac marrow shielded. There was immediate depression until day 18 when recovery began. The rate of recovery was similar to that follwoing local irradiation. The low concentration was equal to the net increase in number of cells during the recovery period following local irradiation and prior to total body irradiation.

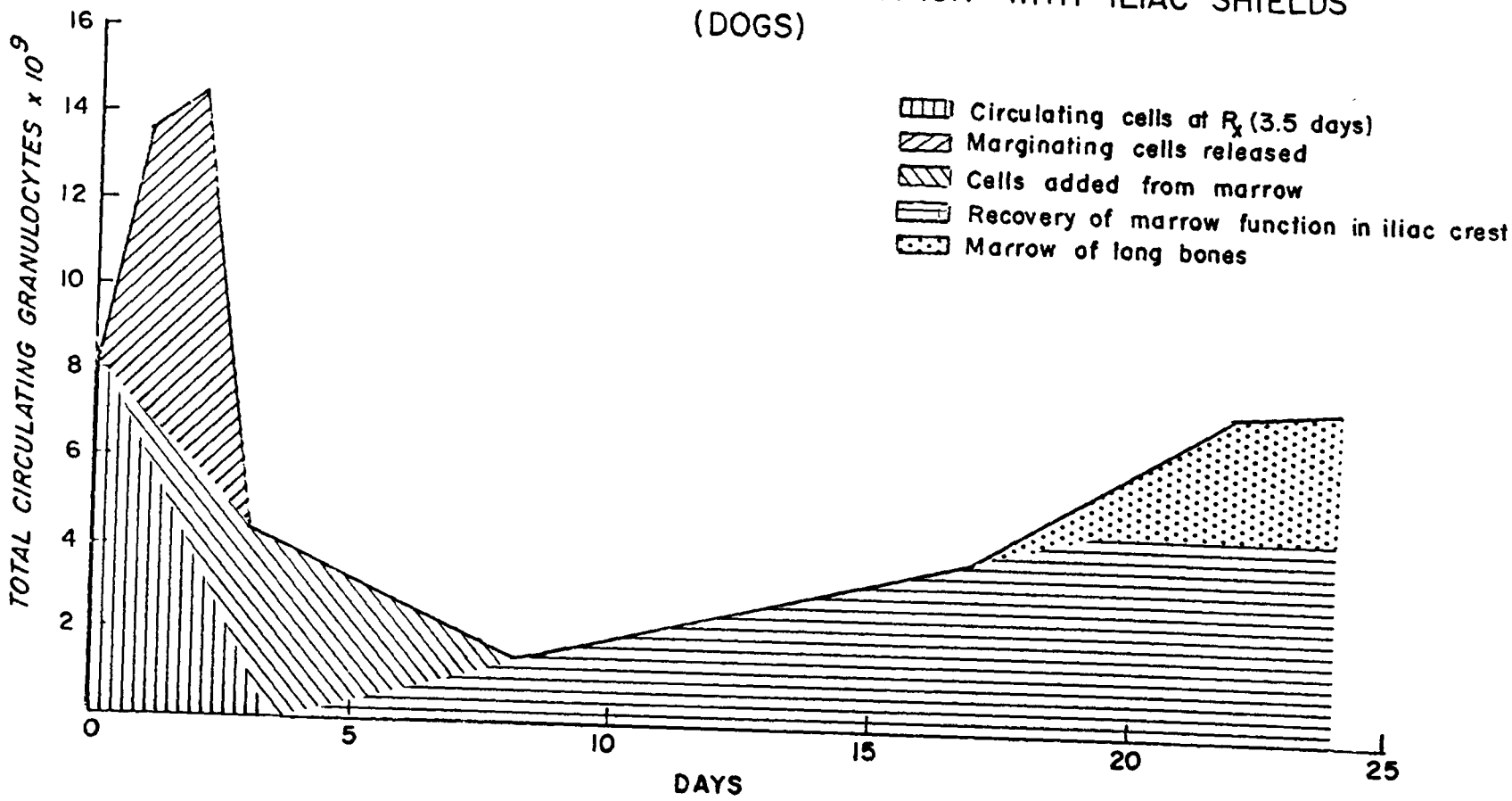
Both experiments demonstrated early recovery of iliac crest marrow. This may be the result of: 1) a large bulk of marrow offering probability th at a large number of cells will survive irradiation, 2) the protected location of iliac crest marrow which may decrease amount of exposure, 3) some difference in sensitivity of marrow cells located in iliac crest as compared to the marrow found in other bones.

The summation of these variables accounts for the behavior pattern of peripheral granulocyte count following total body irradiation (figure 6).

Using cellular tagging techniques, the relation of kinetics of cellular

Fig. 6

EVENTS INFLUENCING THE PERIPHERAL GRANULOCYTE CONCENTRATION FOLLOWING TOTAL BODY IRRADIATION WITH ILIAC SHIELDS (DOGS)



elements of marrow to peripheral blood have been studied.* The compartments or stages of development in the precursor granulocytic elements found in bone marrow of dogs have been described in terms of number of cells and time spent in each compartment. Using this information and observations on our own experimental animals, a scheme of granulocyte proliferation was formulated.

This is a self-perpetuating system at the stem cell level. As a result of a single division in a 48 hour period there are 2 daughter cells. One returns through division to stem cell, the same as the parent cell. The second daughter cell divides again and in 48 hours there are two new daughter cells. These cells start the transformation process to become myeloblasts. This is followed by successive single divisions in each stage of development,--myeblasts, promyelocytes, and generative myelocytes. After the generative myelocyte stage, there is differentiation through the transitional myelocyte, metamyelocyte, marrow band and marrow segmented neutrophil stages without further division. A single stem cell will release 16 mature segmented neutrophils into circulation every other day. There is another group which will release mature segmented cells into circulation on alternate days. In the steady-state proliferation scheme, the self-perpetuation is shown to be the result of self-duplication at the stem cell level. To obtain a linear increase in the number of stem cells (non-steady state), these cells need only to

*Lajthan, L.F. On DNA labeling in the study of the dynamics of bone marrow cell populations. The Kinetics of Cellular Proliferation. Grune & Stratton, New York 1959. F. Stohlman, Jr. Ed. p. 173-187.
Bond, V.P., Fliedner, T.M., Cronkite, E.P., Rubini, J.R. and Robertson, J.L. Cell turnover in blood and blood-forming tissues studied with tritiated thymidine. Ibid. p. 188-200.
Patt, H.M., and Maloney, M.A. Kinetics of neutrophil balance. Ibid. p. 201-207.

reduplicate one additional time. The result is four stem cells. One cell returns through division the same as the original parent cell; one continues the normal transformation to the myeloblast stage. The remaining two cells divide as the parent cell of the steady-state cycle. The result of the division of the altered stem cell cycle is the creation of a steady-state cycle every other day. As each myelobalst results in eight mature granulocytes in the circulation, the ratio of cells being added to the peripheral circulation can be determined. Application of this modified scheme of cell division to the experimental animals results in an increased in the accumulated ratios proportional to the observed cell counts.

Utilizing this modification, the recovery rate in terms of circulating granulocytes can be determined with indications to the apparent stage of development in the marrow at the time of irradiation. Comparison of the original impression of events influencing the peripheral granulocytes to the quantitative recovery indicates the original hypothesis is supported by quantitative considerations. The behavior pattern is very similar. The only part which does not follow the early impression is the quantity of cells surviving the direct effects of radiation. Preliminary calculation indicates that the number of cells in each compartment of development surviving the direct effect of radiation can be determined, evaluated quantitatively, and correlated to the amount of irradiation administered.

Application of these findings to the expected behavior in humans was undertaken. The number of cells (compartment size) for 1) myeloblasts and promyelocytes, 2) myelocytes, 3) metamyelocytes and bands, is in the ratio

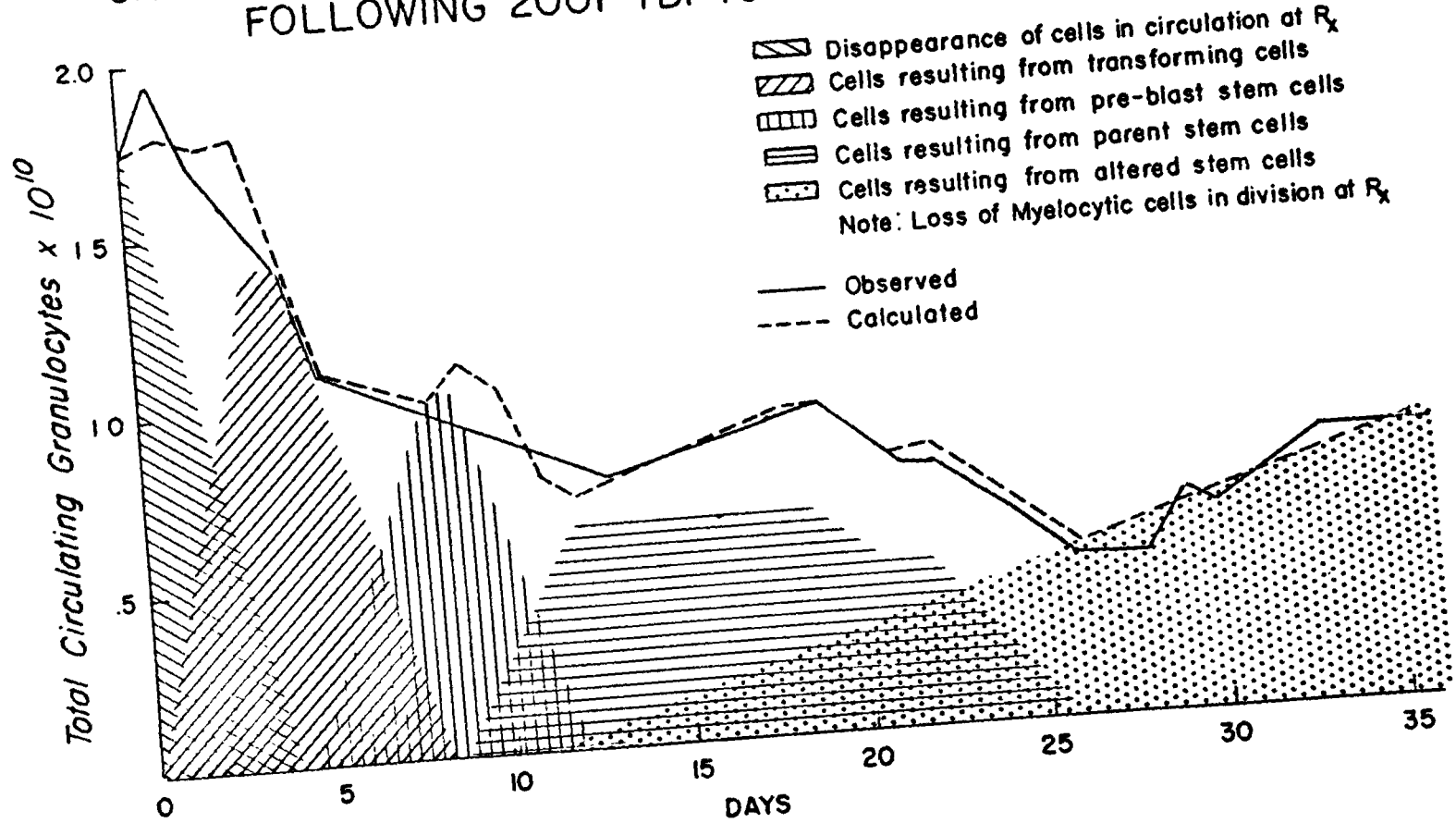
of 1 : 4 : 9. The specific ratios in two groups of dogs and the ratios found in human marrow are approximately the same. This would suggest that there is little difference in the schematic cell division in dogs and humans. The longer time required for humans to demonstrate signs of marrow recovery may be due only to differences in compartment time and size. Calculations show this increased time and size may be the same in all the compartments. Assuming a homomorphogenic division of cells and utilizing results of the ratio of cells in each compartment and the appearance of the tritiated thymidine labeled cells in circulation, the time spent in each compartment was determined. This was found to be longer in humans than in dogs and the increase was a constant factor. The individual time spent in the generative myelocyte and transitional myelocyte compartments could not be determined.

A scheme for the proliferation of human marrow was formulated for the steady-state and non-steady state. The division of cells was similar to that obtained for dogs. Using this scheme to obtain quantitative interpretation of the postirradiation behavior in the concentration of granulocytes, an estimate of the stages of marrow cell development at the time of irradiation can be obtained. Figure 7 shows the quantitative estimate of cells compared to the observed peripheral count following a single exposure of 200r total body irradiation. Depression and recovery are analyzed as follows:

1. Granulocytes which were present in the peripheral circulation show not evidence of direct destructive effect from 200r (single dose) total body irradiation. Survival time is equal to the pre-irradiation value.
2. Granulocytes in marrow which are in the transformation compartment continue on to maturity.

Fig. 7

CALCULATED RECOVERY OF TOTAL CIRCULATING GRANULOCYTES COMPARED TO OBSERVED RECOVERY FOLLOWING 200r TBI TO A HUMAN



3. Approximately 20% of cells in the immediate premyeloblast stage are lost. The rest continue on to normal maturity.

4. Fifty per cent of the parent stem cells are lost directly to the effect of irradiation; 7% of the stem cells are altered to form the non-steady state recovery cycle. The remaining parent stem cells survive and continue normal division but only until the 18th and 19th day. These cells apparently are unable to continue division and are lost.

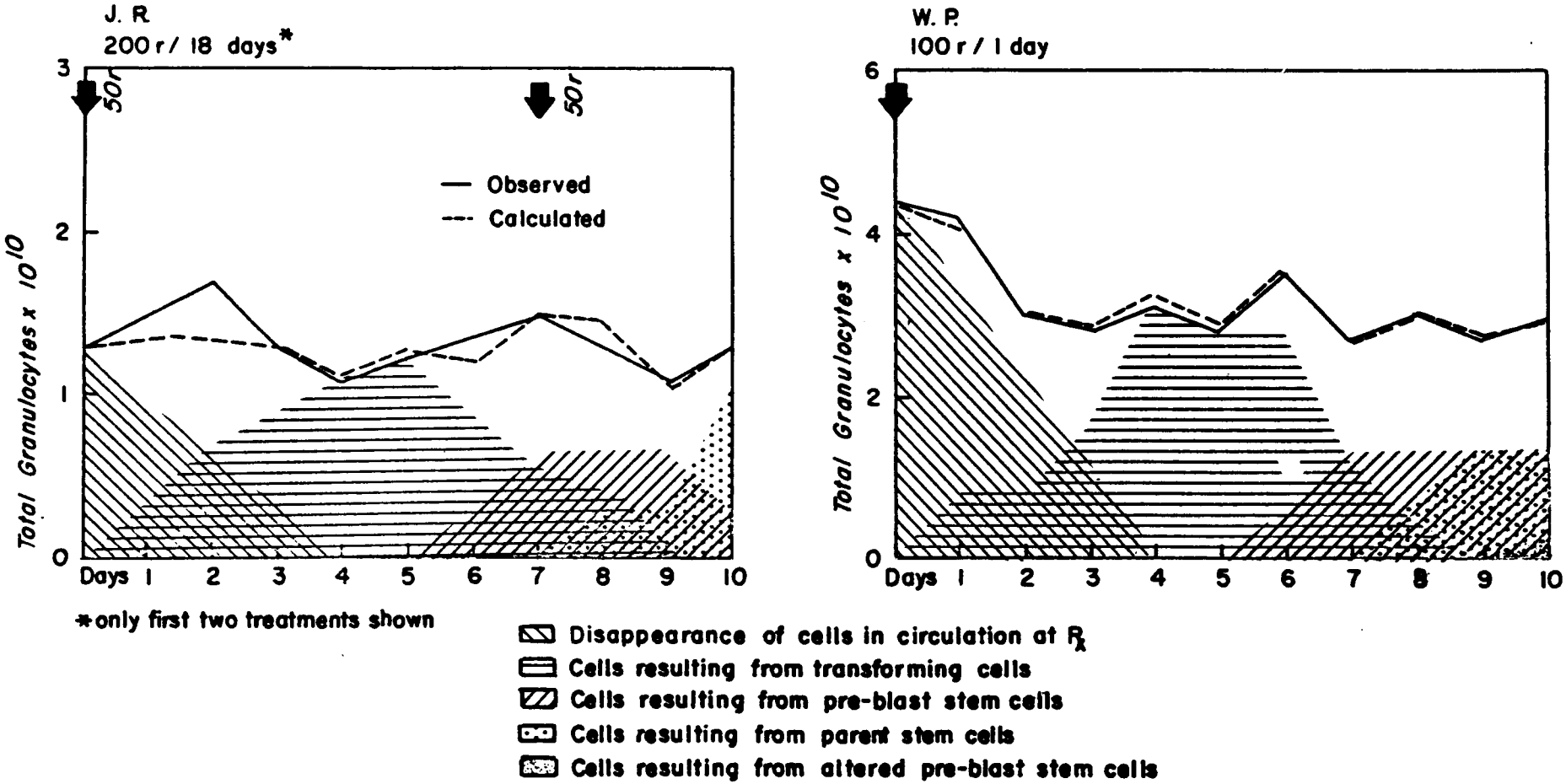
5. All the precursor cells, i.e., myeloblasts, promyelocytes and generative myelocytes, are lost to the direct effect of irradiation.

Only those cells produced by the altered cycle are capable of continued division leading to eventual recovery.

This scheme of granulocyte proliferation provides a basis for predicting the cell concentration during the recovery period following various amounts of total body irradiation. Figure 8 shows the observed and the calculated granulocyte response in two patients (Nos. 105 and 100, Part I) following total body exposures of 50r and 100r respectively. Following 50r, neither the calculated nor the observed response show depression. Since there was no loss of cells, the slight increase on day 2 must be attributed to delayed release of some cells from marrow. Similar fluctuations have been noted among several other patients receiving total body irradiation and also among normal individuals. Further study may indicate that this increase is a characteristic of the individual. Following 100r, the calculated and observed response are almost identical; depression is slight but definite and calculations indicate that about 4% of parent stem cells are lost, along with some dividing cells of the transforming compartments. The graphs

Fig. 8

CALCULATED RECOVERY OF TOTAL CIRCULATING GRANULOCYTES
Compared to Observed Recovery Following 50r and 100r TBI to Humans



suggest that 50r total body irradiation produces little or no change in normal granulocyte proliferation, and only slight depression follows 100r. Since granulocyte proliferation represents a rather sensitive index, it seems likely that radiation change in other systems would be comparable following similar exposures.

These studies in granulocyte proliferation also provide a basis for investigating the value of bone marrow transplants. When severe bone marrow depression occurs following total body exposure, attempts are made to replace aplastic marrow by means of infusion of viable marrow cells. It is believed the infused marrow elements find their way to favorable marrow sites and by division and multiplication, repopulate marrow cavities and restore bone marrow function (Trentin, J. Whole body x-ray and bone marrow therapy of leukemia in mice. Proc. Am. Assoc. for Cancer Research 2:256, 1957b).

Animal experiments dealing with high level irradiation with shielding of iliac crest marrow gives some indication of the amount of viable marrow required for protection. Although scattered radiation may reduce the number of viable cells in the iliac crest, the recovery pattern indicates that sufficient viability is retained for adequate protection. The recovery pattern also gives some indication of the time required for these cells to be released into circulation, i.e., the first clinical evidence of beginning recovery.

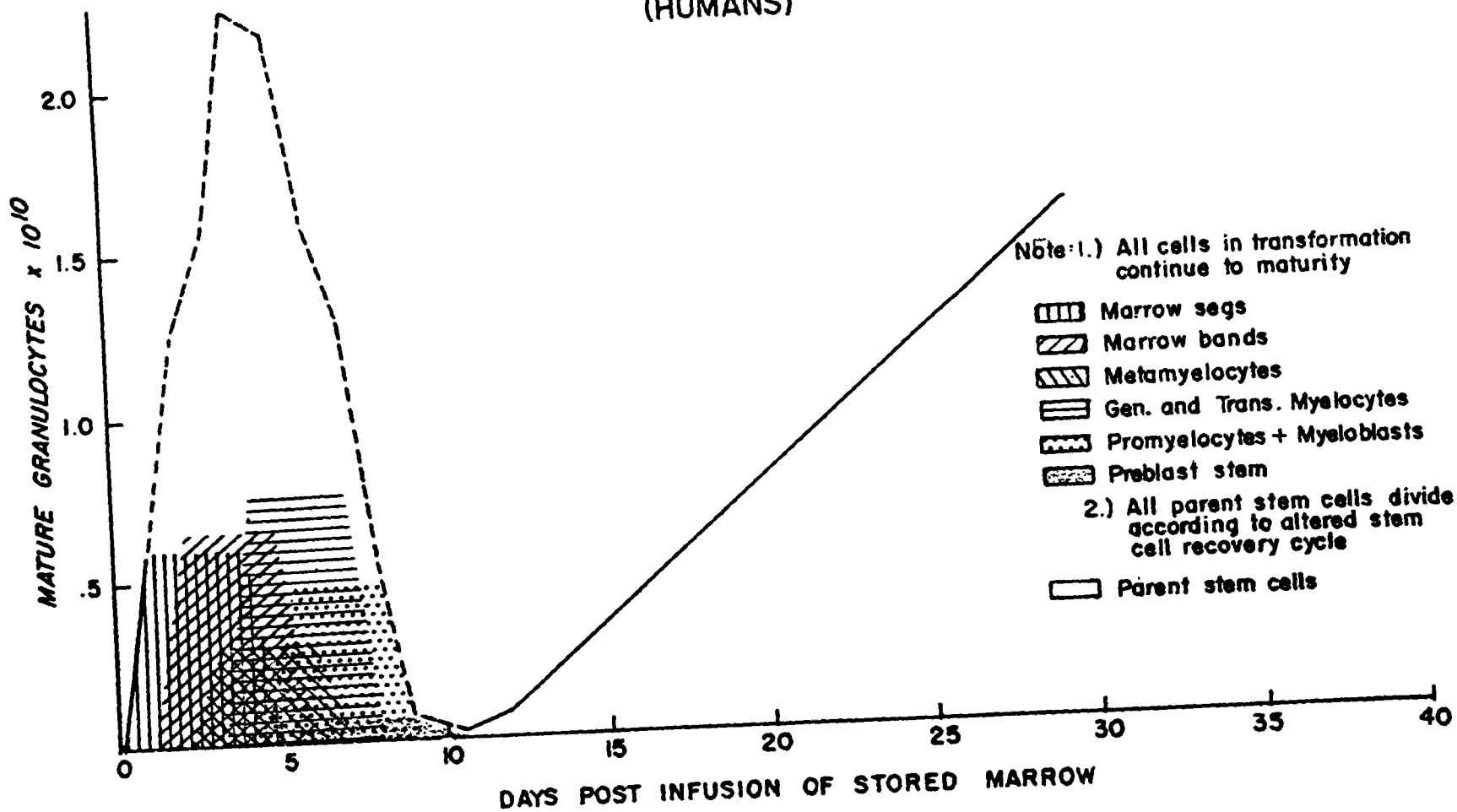
With the methods of processing and storage used in this laboratory, about 90% of marrow cells are viable at the time of re-infusion and, normally, approximately 2/3 of these are granulocytes in all stages of development. If each cell implanted is established in marrow sites on the day of infusion and maintains its ability to divide and progress through steps of maturation,

the minimum time required for beginning recovery would be 11 days (see Fig. 9). Those cells in the final compartment before maturity would be released in circulation on day 1; since the circulating life span is 4 days, these would be lost on day 5. This progression of various types of cells, maturing, released in circulation and lost, continues until the histiocytes (stem cells), proliferating through the non-steady state scheme, appear in circulation and signify beginning recovery. At this rate of recovery, a safe concentration of granulocytes would be achieved on day 20 or 21 following bone marrow infusion.

Following therapeutic total body irradiation in single exposures, the time of greatest hematopoietic depression occurs between days 30 and 35. If severe or critical depression is anticipated, and if calculations are correct, the infusion of bone marrow would be most beneficial if given prior to the 10th post-irradiation day. This would permit granulocytes to proliferate to a safe level at a time when irradiated marrow reached its lowest depression. While this plan seems reasonable in theory, it must be confirmed by practical experience (see Part V). If correct, it will provide a basis for study of a plan for re-infusion of bone marrow following protracted exposures of total body irradiation.

Fig. 9

INFLUX OF MATURE GRANULOCYTES FOLLOWING INFUSION OF 32 x 10^9 VIABLE BONE MARROW CELLS (HUMANS)



Part V. Future Plans

Clinical observations will be continued on all patients requiring therapeutic total body irradiation. When possible and feasible, the observations will be extended to include: 1) processing, storing and retrieving of clinical data with automatic data processing and computing equipment, 2) cardiodynamic observations and 3) total body composition studies. These extensions are planned in collaboration with Dr. William Spencer and his group at the Texas Institute for Rehabilitation and Research. With equipment and personnel available, a comprehensive protocol was drawn up to include all possible clinical and laboratory studies for periodic assessment of the effect of total body irradiation on all systems, and on the patient as a whole (see attached protocol). In order to determine the practicality of this extensive investigation, the entire procedure was carried out on a patient who received large abdominal field (30 x 30 cm.) irradiation.

D.K.	Age 74 C/M BUCM 5848
Sept. 1961	Increasing abdominal pain, constipation, and weight loss (70 lbs.) over a period of 3 years
Oct. 1961	Laparotomy disclosed large, inoperable mass involving all intra-abdominal structures and peritoneum Biopsy - Path. report: Cancer of tail and body of pancreas with multiple metastatic implants.
12/10/61	Admitted to Texas Institute for Rehabilitation and Research Tests conducted according to protocol Radiotherapy, large abdominal field (30 x 30 cm.) 300r/1 day, 2 m v Tests continued and repeated according to protocol
12/20/61	Some abdominal pain with signs of partial duodenal obstruction with bleeding, edema of left extremity due to lymphatic obstruction. Discharged, to return in 2 weeks for repeat studies and additional radiotherapy
12/28/61	Died of disease

Comment:

Because the tests outlined in the protocol are used routinely to assess the progress of rehabilitation among disabled patients, the poor condition of the patient D. K. offered no handicap to successful completion of the studies. Work-up prior to radiotherapy required approximately 3 days and repeat studies were carried out following exposure and on day 7; arrangements were made for final tests 3 weeks post-irradiation with follow-up observations and additional studies to continue as long as possible. This would allow periodic assessment and comparison; changes occurring during this time would provide some indication of the value of such tests for total body irradiation patients. In this case, the studies planned at 3 weeks and thereafter could not be carried out. However, several findings revealed during continuous recording and by pre- and post-irradiation studies seemed particularly significant.

Processing, Storing, and Retrieving of Clinical Data with Automatic Data Processing and Computing Equipment.

Clinical charts and records tend to fall between inadequate for the purpose of long term studies, and so voluminous as to preclude a perusal for extraction of significant data. This area has lagged in the application of automatic data processing and computer methods for many reasons, but principally perhaps because it seemed that clinical history and observations were not susceptible to such recordation. Under the direction of Dr. William A. Spencer and Dr. Carlos Vallbona, the clinical research records of the Texas Institute for Rehabilitation and Research have been developed with a view toward data retrieval and computer analysis of the quantitative information that they contain. The pattern of the work is well established and adaptation

to the time course study of radiation effects in humans is feasible.

As an example, records of the different observations on the patient D.K. are appended. This methodology can as readily be applied to the entire protocol which is also appended.

Cardiodynamic Observations

Intermittent or continuous recordings of respiratory frequency and heart rate can be carried out before, during, and after irradiation. The technique used (cardiotachometry) measures the interval between heart beats; thus, small differences or variations of short duration are identified that otherwise would be overlooked.

The normal influence of respiration on instantaneous cardiac rate can be readily observed. Variations superimposed on these from other regulatory or controlling mechanisms are also detectable. These may be due to autonomic influences of central origin. Some correlation with nausea seems to exist (see tracings A, B, and C) and these measurements may provide an objective test for what has been a subjective element of "radiation sickness."

Tracing A reveals a beat by beat arrhythmia that was present during most of the recording. There are two short periods (a and b) where the chronicity of the heart beat is regular. The origin of this arrhythmia was probably central since the ECG recorded simultaneously failed to reveal a change in pace maker.

Tracing B shows regular chronicity of the heart beat and complete absence of the respiratory fluctuations of the heart rate that occur in health. At point "c" there was a fluctuation of deceleration of the heart rate that preceded an episode of nausea. Vagal stimulation associated with nausea is of particular interest since it occurred prior to radiation exposure. If vagal

HEMATOLOGY

A-60 | X-61

DATE	TIME	HGB	RED CELL SERIES: RBC	ERYTHROCYTE: POLYCHROMASIA	ERYTHROCYTE: ANISOCYTOSIS	ERYTHROCYTE: MICROCYTIC	ERYTHROCYTE: HYPOCHROMIA	ERYTHROCYTE: MACROCYTIC	ERYTHROCYTE: STIPPLED	ERYTHROCYTE: OTHER	ERYTHROCYTE INDICES: MCH	ERYTHROCYTE INDICES: MCV	ERYTHROCYTE INDICES: MCHC	RETIC COUNT %	WHITE CELL SERIES: WHITE BLOOD CELLS THOUSAND	SEGS	BAND FORMS	LYMPHS	MONOS	EOS	BASOPH	IMMATURE WHITE CELLS	SED RATE MM/HR	PLATELETS	BLEEDING TIME MIN	COAGULATION TIME MIN	CLOT RETRAC TIME MIN	CLOT RETRAC %	EOSINOPHIL COUNT PER CU MM	EOSINOPHIL SMEAR %	ERYTHROCYTE FRAGILITY: % NaCl INITIAL	ERYTHROCYTE FRAGILITY: % NaCl COMPLETE	L E CELL PREP *	PLATELET COUNT THOUSANDS	SICKLE CELLS %
121161	810	7.4	23	3.24										4.7	10.4	74		21	04	03				A		12.5			105					
121261	830	8.5	27												10.1	65	02	30	03					A										690
121361	1100	9.0	27												11.0	66	01	26	06	01				A										580
121461	800	7.6	24		2									8.2	8.5	67		10	23	01				A										514
																										18.7								765
121561	800	7.4	25	3.24	2						23	77	3	5.2	7.6	86	02	6	04	02				A	.4									268
121661	800	7.4	23	2.02							36	133	2	1.9	11.1	90	03	6	01										72					
																													75					
121761	800	10.6	34	4.12							26	83	32	2.7	9.0	93		6		01														323
																																		78
121861	1500	7.1	21	2.48							29	85	34		7.7	85	01	9	05					A					50					
121961	800	7.1	22	2.09							34	05	33		4.2	91	02	5	02					A										273
																																		272

* 0 = NEG 1 = POS.

BLOOD CHEMISTRY

DATE		SOURCE	CONDITION	TOTAL PROTEIN G%	ALBUMIN G%	GLOBULIN G%	UREA N MG%	GLUCOSE MG%	CHOLESTEROL MG %	AMYLASE UNITS	PBI MCG %	TRANSAMINASE UNITS	URIC ACID MG %	CREATININE MG %	ACID PHOSPHATASE UNITS	OTHER	CO2 CONTENT MEQ/l	CLORIDE MEQ l	SODIUM MEQ/l	POTASSIUM MEQ/l	MAGNESIUM MEQ l	CALCIUM MEQ/l	PHOSPHATE INORGANIC MG %	PH

```
121161  VF 6.5       011008301580058     6.6        105145 4.0
121161                                              LIPIDS 1ST 400 2ND 357
121361  ROUTINE GASTRIC ANALYSIS VOL 20CC
121361  COLOR/BROWN ODOR/SOUR
121361  MUCUS/PRESENT QUANTITY/5.6CC
121361  QUALITY/MODT. VISCOSITY
121361  SALIVARY CONTAMINATION/ MOD AMT.
121361  PH/3.6 UNITS BILE/ABSENT
121361  FREE HCL 4 UNITS/100 MLG
121361  TOTAL ACIDITY 27 UNITS/100 MLG
121461  VF           034007201570044            D 1ST LIPID 389 MG%
121561  VF           0460100    0106            PIDS 1ST LIPID 371 MG%
121661  VF           0470088    0060
121761               0480076    0172
```

12512KIMBLE DFF

BACTERIOLOGY

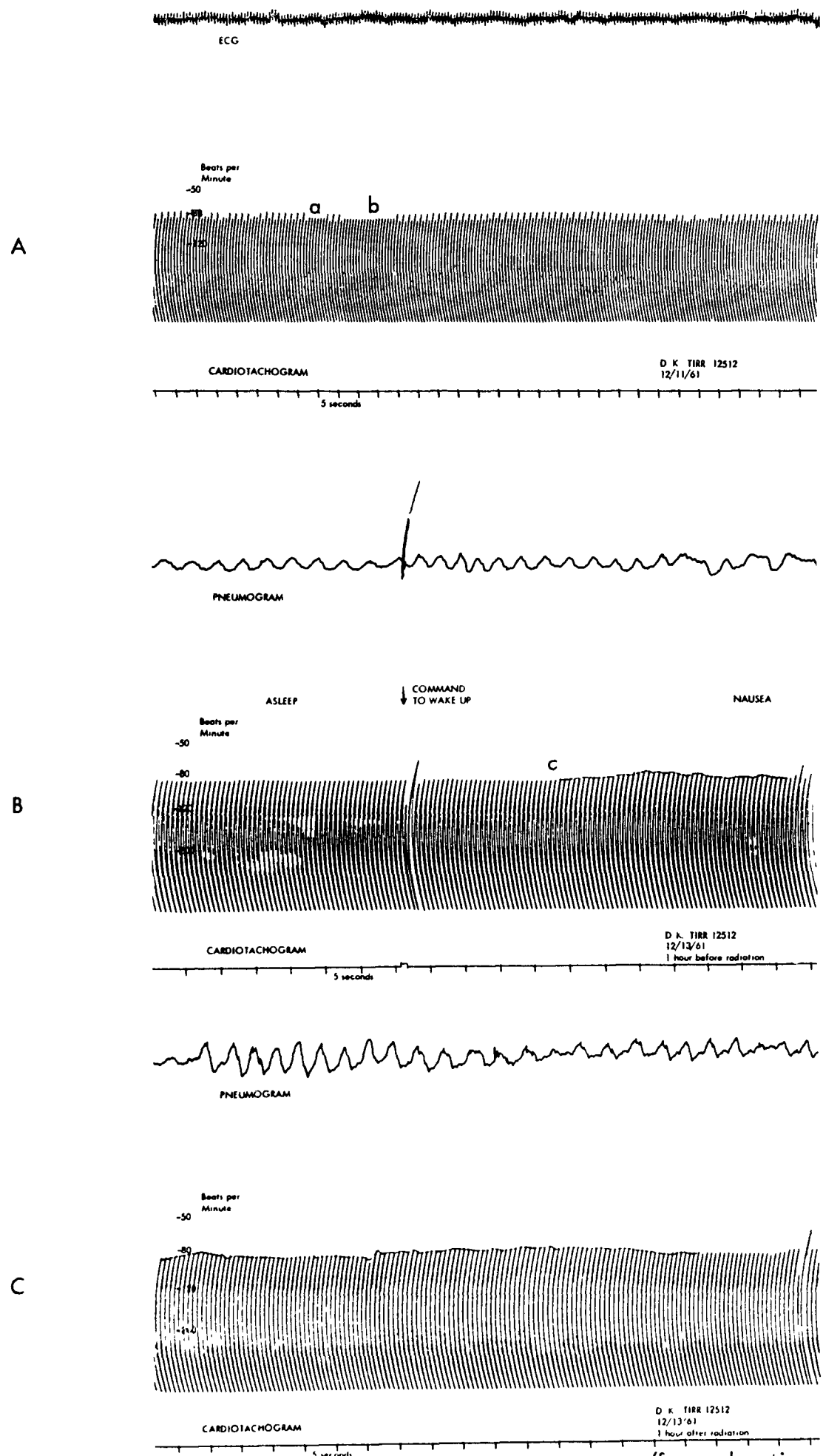
DATE MO	DAY	YR	OUT PATIENT	TIME	TYPE SPECIMEN	STUDIES REQUESTED	DRUG	REPORT OF SMEAR: GM + COCCI	GM + DIPLO	GM + RODS	GM − COCCI	GM − DIPLO	GM − RODS	AFB	AF CONC	YEASTS OR FUNGI	PUS CELLS	CULTURE GROWTH	SOURCE OF CULTURE	ORGANISMS FOUND	SENSITIVITIES: PENIC	STREP	TETRACYC	DECLOMYC	CHLORO	ERYTHRO	NOVO	KANA	VANCO	BACI	POLYMYXIN	COLIMYCIN	RISTO	FURAD	TRI SUL	GANTRI	KYNEX	STAPH	MAND	OTHER	OTHER	*CODE
12	11	61		1215	10	23												P	10		1	2	1	2	2	1	1	2	1	1	3											62
																		P	10	08A																							63
																		P	10	09G																							64
12	20	61		800	10	23												P	10	03A		2	1	2	2					1	1		2	3	3	3					65
																		P	10	06C		1	2	2	1					3	3		2	1	1	2					63
																		P	10	09A	3	1	2	1	2	1	2	2	3	1	2										64
																		P	10	08NHC	1	1	1	2	2	3	2	2	1	3	2										65

12512KIMBLE DEF

*CODE = 62, 63, 64, 65, 66

PAT NO	DATE CHG	TIME	TIME	SPECIMEN	BSP	BILIRUBIN TOTAL	BILIRUBIN DIRECT	CEPH CHOL FL	PHOSPHATASE ALK	PROTHROMBIN	THYMOL TURBIDITY	TRANSAMINASE	UROBILINOGEN	URINALY	UROBI INOGEN	FECAL	DATE ANAL GREG	DATE ANAL CENT
12512121161		00815		B	008	1.0				040				-				23
12512121161		00816		B	PROTHROMBIN 20 SEC CONTROL 15SEC									-				23
12512122061		00800		B		3.3			7.2035			0014		-				12216123
12512121761		00800		B										-			050012206123	
12512121661		00800		B										-			050012206123	
12512121561		00800		B										-			043012206123	

PAT NO	DATE GREG	CHG	TIME	TIME	CCAFSOONSAETT OOBATCVETSNRR LNNTACACRCTII OSO RU AOAECC RIR CLPTNRRHH SM HTAOGIO T RR SBTS EC BA S IRP NO L ATLUII CN OPMEUSCR YT ORERM H DOR BV	H Y M E N N A N A	EEG NNI DDA AAR D HCI IOA SLL TIA O M B	2
12512121161	01215				1			12116112
12512121461	01530				4			12146112
12512121561	01435				4			12156112
12512121961	01130				4			12196112
12512122061	00800				4			12206112



(for explanation, see text)

stimulation is an effect of irradiation, then this would be recorded for all patients receiving total body exposure whether or not symptoms of nausea and vomiting developed. If detected only in those patients who developed nausea and vomiting, then the stimulus must be attributed to some other influence, i.e., psychologic, individual sensitivity, etc.; if detected in patients without manifestations of nausea, this test might confirm that vagal stimulation is a direct effect of irradiation. Additional evidence might be provided by observing the effects of irradiation directed to the site of origin of the vagus nerve.

Tracing C brings into evidence for the first time small but visible oscillations of deceleration-acceleration that are concomitant with respiratory movements.

These observations will be carried out on patients receiving total body irradiation and those receiving irradiation to the entire brain and brain stem before, during, and after treatment.

Total Body Composition Studies

Total body water, extracellular water, and intracellular water are variables susceptible to measurement that may reflect a response to injury at the cellular level where radiation effect is probably exerted.

Table 4 shows body composition estimates on the patient D.K. before and after irradiation. As will be noted, there was reduction in total body water with changes in ratio of components. Increase in extracellular water with decrease in intracellular water could not be attributed to edema since this would be reflected by increase in total body water. Since this was not the case, the change in ratio could be due to effect of irradiation at the cellular level.

Table 4

Body Composition Estimates Before and After Irradiation
(D.K. W/M Age 78)

	Theoretical	Before	After
Body Weight	63.2 kg.	64.3 kg. (141.5 lbs.)	58.2 kg. (128 lbs.)
Height		167.6 cm. (66 in.)	167.6 cm. (66 in.)
B.S.A.		1.73 m^2	1.66 m^2
Total Body Water			
% Body Weight	58.6% $\pm$ 3.50	54.7%	57.6%
Absolute		35.2 L.	33.7 L.
Extracellular Water			
% Body Weight	39.9%	34.5%	39.9%
Absolute		22.2 L.	23.2 L.
Intracellular Water			
% Body Weight	32.0%	20.2%	17.7%
Absolute		13.0 L.	10.3 L.
Lean Body Weight			
% Body Weight	80.0% $\pm$ 4.53	76.0%	70.0%
Absolute		48.9 kg.	46.5 kg.

Other quantities such as lean body mass and total fat content, derived from the previous measurements, may be useful in estimations of qualitative irradiation effects and in distinguishing those to be expected in normal individuals from those occurring in patients with advanced cancer. For example, the loss of cell mass which might be expected to occur in irradiating a patient with leukemia or lymphoma may be recognized as such and distinguished from other effects of radiation which might be expected to occur in a normal individual.

Results of this first trial with the all-inclusive protocol indicate that many responses to irradiation of various systems, the patient as a whole, and at the cellular level require further exploration. To determine the nature of these responses, and to eliminate those tests which make no contribution to the investigation, a pilot study is planned. It is felt that 10 patients receiving total body irradiation with studies as outlined in the protocol would offer considerable information. Since this requires admission to the Texas Institute for Rehabilitation and Research with utilization of highly specialized personnel and equipment under the supervision of Drs. Spencer, Vallbona and Cardus, the economic aspects of the study were considered. The total cost for each patient was estimated to be about $2,000.00. This includes approximately 20 days hospitalization, nursing care, and all studies required by the protocol. Funds are available for this project under the present contract and request for additional support is not anticipated.

Animal experiments designed to establish quantitative evaluation of granulocytopoiesis utilizing tritiated theymidine will be undertaken. Observations on the progress of "tagged" cells through each succeeding compart-

ment will be made. Any change in the normal process following total body exposure would be an indication of the sensitivity of each cell type. This information will provide a basis for fairly accurate prediction of degree, time and rate of depression and recovery. A protocol has been drawn up and materials for this work are being assembled. Similar studies following protracted exposures to total body irradiation are expected to provide information on cumulative effects and may indicate the intervals when an exposure could be repeated without undue reaction or depression.

Activities of the bone marrow bank will be expanded to include more patients for aspiration, storage, and re-infusion of marrow. Present techniques will be revised to include: 1) determination of amounts of different marrow elements obtained at aspiration, i.e., fat, platelets, red blood cell precursors, and granulocyte precursors; 2) determination of percentage of each cell type that retains viability after storage; 3) when feasible, tagging marrow cells of humans will be considered so the process of establishment and repopulation can be observed and followed; 4) study of the effect of protracted total body irradiation on re-infused marrow in order to determine whether marrow should be returned following a course of protracted exposures, or returned in divided amounts at some specified time following each exposure.

A bibliography consisting of publications dealing with total body irradiation has been assembled. Abstracts have been prepared and a method of classification of this material for ready reference is being studied. Because of the many aspects involved, several broad categories such as total body exposure to humans, to animals, and in vitro studies, with many sub-classifications of dosimetry, protection and response would be useful. Cross-

reference by means of a punch card system is being considered. Because new articles appear constantly, this is a continuing project and it may be necessary to limit additional abstracted material to those which add new information and record others by author and title only. Details of this work with analysis of the material will be included in the next progress report.

Total Body Irradiation

Protocol December 1961

This protocol is intended to be as all inclusive as possible and all information collected will be subject to analysis by means of the data processing machine in collaboration with Dr. William Spencer's group at the Texas Institute for Rehabilitation and Research. An attempt will be made to obtain all tests on all patients receiving therapeutic total body irradiation but changes in the protocol will be permitted when indicated by the patient's condition or by lack of facilities. However, any change must be justified in writing in the patient's chart. Patients hospitalized at the Rehabilitation Center will undergo additional tests as outlined by Drs. Spencer, Vallbone and Cardus (see last page of protocol). One member (resident or staff) will be responsible for supervising the care of the patient and for obtaining laboratory studied throughout the hospital course. Prior to administration of total body irradiation, a staff meeting will be held to determine changes in the protocol, method, amount and time of exposure. A duplicate chart on each patient will be made and filed at Baylor for reference, analysis, and comparison. This will include a record of history and previous treatment, progress notes, laboratory data, and arrangements for follow-up.

Protocol

1. Complete history and physical examination including:
    a. Summary of previous treatment (radiation and chemotherapy)
    b. Measure and record in cm. visible and palpable lesions
    c. Photography of patient if indicated
    d. Body measurements for integral dose (Bob McTaggart)

2. Bone marrow aspiration for banking

3. Laboratory work

   a. X-rays - include chest x-ray on all patients
      bone survey on tumors with bone metastases
      additional x-rays as indicated
      measure and record in cm. all measureable lesions

   b. Hematology studies
      C.B.C. (WBC, Diff., RBC, Hgb., Plts., Eos., Retics.)
      Sedimentation rate
      Bleeding time
      Clotting time
      Bone marrow, diagnostic (iliac crest & sternum)

   c. Urine
      Routine urinalysis
      PSP
      24 hour urine creatinine

   d. Blood chemistries
      Blood sugar
      Serum amylase
      Serum lipase
      Cholesterol
      Lipids
      Na, K, CO_2 combining

      Blood volume

      Liver function studies, including
      B.S.P.
      Alkaline phosphatase
      Serum bilirubin
      Thymol turbidity
      SGOT
      SGPT
      Prothrombin time
      Serum total protein & A/G ratio
      Serum electrophoresis
      Uric acid
      B.U.N.
      Lactic acid dehydrogenase

   e. Bacteriology
      Urine culture
      Stool culture
      Blood culture

f. Other studies
Stool for occult blood
Duodenal biopsy
Rebuck test
EEG
EKG
Nerve conduction
Psychological and intelligence tests
Serum for Dr. Luzzio (Bob McTaggart)

The following tests will be done daily.
C.B.C., including W.B.C., Diff., R.B.C., Hgb., Eos., Plts., Retics.
Blood sugar (daily for 1st 4 days, then weekly)
Serum amylase (daily for 1 week, then weekly)

The following studies will be done on the day following total body irradiation and at weekly intervals until the patient is released.

1. Measure and record in cm. all visible and palpable lesions
2. Measure and record in cm. all measureable x-ray lesions
3. Sedimentation rate
4. Bleeding time
5. Clotting time
6. Routine urinalysis
7. PSP
8. 24 hr. creatinine
9. BSP
10. Alkaline phosphatase
11. Serum bilirubin
12. Thymol turbidity
13. SGOT
14. SGPT
15. Prothrombin time
16. Serum total protein and A/G ratio
17. Serum electrophoresis
18. Uric acid
19. Blood sugar
20. Serum amylase
21. Lactic acid dehydrogenase
22. Serum lipase
23. Cholesterol
24. Lipids
25. Na, K, & CO_2 combining
26. Urine culture
27. Stool culture
28. Blood culture
29. EEG
30. EKG
31. Nerve conduction

The following tests will be done as follows:

1. Diagnostic bone marrow aspiration, iliac crest & sternum, every other week
2. Blood volume, every other week
3. Duodenal biopsy, 3rd day post T.B.I. and at 2 weeks
4. Rebuck test - 7 days after last exposure
5. Psychological and intelligence test at 3 weeks and 6 weeks.

The following additional studies are to be done if the patient is admitted to Texas Institute of Rehabilitation and Research. The times at which these tests will be done is to be decided at the pre-treatment meeting.

a. Body composition studies
   Evans blue
   Thyocyanite
   Antipyrine

b. Urine corticoids

c. Cardiovascular studies
   ECG - dynogram
   Passive tilt
   Cardiotach
   Impedance
   Ergometry

d. Respiratory studies
   B.M.R.
   ph, pCO_2, pO_2
   Compartments
   Distribution
   M.B.C.
   Compliance

e. Muscular studies
   Nerve conduction
   Isometric contraction
   Grip strength

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